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# Porphyrin-Based Photocatalytic Lithography

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**ABSTRACT:** Photocatalytic lithography is an emerging technique that couples light with coated mask materials in order to pattern surface chemistry. We excite porphyrins to create radical species that photocatalytically oxidize, and thereby pattern, chemistries in the local vicinity. The technique advantageously does not necessitate mass transport or specified substrates, it is fast and robust and the wavelength of light does not limit the resolution of patterned features. We have patterned proteins and cells in order to demonstrate the utility of photocatalytic lithography in life science applications.

**KEYWORDS:** microarray, surface modification, porphyrin, photocatalysis, lithography, patterning

## 1. Introduction

Our research examines innovative photocatalytic techniques for patterning surfaces, as well as the development of novel hybrid materials. We seek to engineer dictated chemical patterning and host response. Our long-term goal is the rapid, reproducible and inexpensive patterning of surface arrays with nanometer-scale features. Deterministic collection and organization of proteins, DNA, viruses and cells into ordered arrays holds enormous potential across multiple disciplines, including materials science, synthetic chemistry, biology and synthetic biology (presentation of nanoscale ligands), as well as medicine.

Several techniques for micron- or larger-scale patterning of chemistries for biomolecular and other purposes currently exist.<sup>1</sup> These include photolithography<sup>2-4</sup>, microcontact printing<sup>5-7</sup>, etching with incorporation of elastomeric stencils<sup>8</sup>, and selective molecular assembly patterning (SMAP,<sup>9,10</sup>).

Photocatalytic patterning using metallic oxide catalysts has also been reported<sup>11-13</sup>. These publications utilize TiO<sub>2</sub> as a photocatalytic semiconductor activated with UV energy to degrade underlying chemistry. They do not report use of photocatalytic patterning techniques in the study of biophysical processes.

This article presents initial results using porphyrin photosensitizers for photocatalytic patterning. Photocatalytic patterning with photosensitizers represents a versatile new method for patterning surface chemistry with simple, variable wavelength energy sources, such as a light-emitting diode (LED) flashlight. Advantageously, the technique does not require photoresist; is inexpensive, fast and robust; primarily operates in the molecular, as opposed to the physical, domain; can accommodate various mask materials, chemistries and substrates; is not limited by mass transport; and is capable of patterning from the macro- to the nano-scale.

To demonstrate the technique, we photocatalytically patterned silane using porphyrins, then covalently grafted a non-fouling background. The patterned substrates were analyzed by Atomic Force Microscopy (AFM) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). Additionally, the patterned surfaces were exposed to protein and cells in order to confirm the robustness of the

patterning technique and the non-fouling background, while illustrating the technique's applicability in life science applications.

Although this publication concentrates on micron-scale results, we note that the wavelength of light used to activate the photosensitizer does not determine or limit feature resolution, as it does in photolithography. As will be described in more detail in future publications, we therefore can pattern at the nano-scale using inexpensive and off-the-shelf broadband, low energy light sources.

## 2. Experimental Section

### 2.1 Master and PDMS mask fabrication

Silicon masters were fabricated by standard photoresist photolithography methods, according to the manufacturer's data sheets. Briefly, a Si wafer was dehydrated in an oven at 200C for 30 min and exposed to hexamethyldisilazane (HMDS; Clariant, Somerville, NJ), which served as an adhesion promoter. Next, AZ1518 photoresist (Hoechst Celanese, Somerville, NJ) was spin-coated onto the wafer to a thickness of 1.8 microns. The wafer was baked on a hotplate at 90C for 1 minute and exposed to collimated UV light through a chrome/ glass mask (Advance Reproduction Corporation; North Andover, MA). The wafer was then developed in AZ 1:1 developer and dried under nitrogen. RIE etching was performed in an etcher (Surface Technology System; Newport, UK) for approximately two min, or eight cycles. Remaining photoresist was removed in acetone, and the etched wafers were cleaned in a Piranha etch comprised of concentrated H<sub>2</sub>SO<sub>4</sub>: 30% H<sub>2</sub>O<sub>2</sub> (5:1 vol/vol%) for 20 min. After etching, the wafers were rinsed thoroughly in ultra pure water (UPW, 18MΩcm) before being blown dry with nitrogen and transferred to Fluoroware™ wafer holders. All chemicals were purchased from Sigma Aldrich (St. Louis, MO), unless otherwise noted.

Next, the silicon masters were exposed to oxygen plasma (Plasma Prep II, SPI; West Chester, PA) at 50 mA, 300 mTorr vacuum. Heptadecafluoro-1,1,2,2-tetrahydrodecyl-1-trichlorosilane (United Chemicals, Bristol, PA) was prepared in anhydrous toluene (0.05 vol%), within a glove box purged with nitrogen. Immediately after preparation, the silane mixture was transferred to a laminar flow hood, and

the substrates were immersed in the mixture for 1 min, which was followed by three toluene rinses of 1 min each. After exposure to the fluorosilane, the masters were baked in an oven for 5 min. at 120C, in order to accelerate covalent bond formation between the silane and the SiO<sub>2</sub> surface.

Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184, Dow Corning; Midland, MI) was prepared in a beaker by adding 1 part curing agent to 10 parts PDMS base. After mixing, the material was degassed until all bubbles were removed. The etched, silane-coated Si masters were placed in polystyrene dishes, and the degassed PDMS prepolymer was poured on top of the Si masters to a thickness of a few millimeters, after which the dishes were placed in a 60C oven for at least one hour in order to cure the PDMS. After curing, the PDMS was peeled off of the Si masters and cut with a razor blade to appropriate dimension for use as PDMS masks. The PDMS photomasks were sonicated in ethanol for 60 min, and then left in fresh ethanol overnight in order to remove any un-reacted monomer. The following day, the cured and sonicated masks were blown with nitrogen and allowed to outgas for another day prior to use.

## 2.2 Silanization

Silicon (cut to approximately 1 cm<sup>2</sup>, <100> from Micralyne; Edmonton, Alberta, CA) and glass coverslip (number2, VWR; West Chester, PA) substrates were contained in Fluoroware™ (Chaska, MN) baskets and sonicated first in UPW, then in 2-propanol, and finally in UPW. Each sonication lasted for 10 min. The substrates then were immersed in a Piranha etch bath comprised of concentrated H<sub>2</sub>SO<sub>4</sub>: 30% H<sub>2</sub>O<sub>2</sub> (5:1 vol/vol%) for 20 min, followed by thorough rinsing in UPW. Substrates were individually blown dry under a filtered nitrogen stream and exposed to oxygen plasma (SPI) at 50 mA, 300 mTorr vacuum. Allyltrimethylchlorosilane (ATC, United Chemicals; Bristol, PA) was prepared in anhydrous toluene (1.25% by volume) in a glove box purged with nitrogen. Immediately after preparation, the silane mixture was transferred to a laminar flow hood, and the substrates were immersed for 5 min, followed by three toluene rinses of 1 min each. The substrates (still contained in

Fluoroware™) were placed in a 120C oven for 5 min at, in order to accelerate covalent bond formation between the silane and the SiO<sub>2</sub> surface.

## 2.3 Photocatalytic Patterning

We have employed numerous porphyrin photosensitizers as photocatalysts and have examined their UV absorption characteristics (Table 1). Chlorophyllin copper sodium salt (ethanol, Sigma Aldrich; St. Louis, MO), Hematoporphyrin IX dihydrochloride (methanol, Frontier Scientific; Logan, UT) and Magnesium Phthalocyanine (ethanol or acetone, Frontier Scientific) were added to solvent at concentrations ranging from 1- 4 mg/ml. A cotton swab dipped in solvated porphyrin was used to coat the previously-described PDMS photomasks with the porphyrin photocatalyst. The porphyrin-coated PDMS masks were blown dry with nitrogen and then placed by hand on top of ATC-coated Si chips or glass coverslips. Controlled patterning and removal of the ATC was achieved by local oxidation via approximately 10 seconds activation of the photocatalyst on the PDMS masks with either 480 nm blue or 660 nm red LED light (LUMEX; Glenview, IL; or Superbright LEDs; St. Louis, MO), or with UV light (Greenspot UV Source, Inc.; Lebanon, IN). In addition, a common flashlight (Restoration Hardware; San Francisco, CA) with intensity peaks at 455 and 550 nm was successfully used to pattern substrates on the order of seconds. Localized patterning and removal of the ATC occurred at locations in close contact to the excited porphyrin on the PDMS photomasks, i.e., elevated areas of the masks that were selectively created from the Si masters. ATC areas positioned under recessed PDMS regions remained intact (Scheme 1). Surfaces then were sonicated in solvent for 1 min and blown dry with nitrogen. Control experiments exposed ATC-coated substrates to PDMS photomasks without porphyrin in the presence of excitation energy (light).

## 2.4 Polymer Grafting

On the ATC-coated silicon or glass substrates, a non-fouling, interpenetrating network (IPN) chemistry of P(AAm)-*co*-EG<sup>14</sup>), was covalently grafted to the SiO<sub>2</sub> regions that retained the ATC layer post-patterning. Briefly, acrylamide (AAm, Polysciences; Warrington, PA) was first photopolymerized

onto the unsaturated allyl silane groups using N,N-methylene-bis-acrylamide (BIS) as a crosslinker, camphorquinone (Polysciences) as a surface-based photoinitiator, and acetone as a solvent. Polyethylene glycol methacrylate (PEG, Polysciences) then was introduced into the AAm layer by swelling the layer in methanol and highly crosslinking with BIS.

IPN-patterned substrates destined to be used in protein or cell-based experiments were exposed to aminopropylsilane (United Chemical), in order to create adhesive regions on the freshly patterned (bare) regions of silicon or glass. Substrates then were stored in dessicators until use.

## 2.5 Optical Microscopy

Surface patterning was monitored at each step of the patterning process by exposing the patterned substrates to water vapor<sup>15</sup> and acquiring images with a Nikon D100 camera mounted on a reflectance-based Nikon Labophot 2 microscope. A few images were acquired in quick succession after introduction of water vapor, in order to view the differences in surface energy between the patterned and background substrate regions. The same microscope/camera combination was used to image patterned eukaryotic HeLa cells (see below).

Detection of FITC-labeled neutravidin on surfaces exposed to fluorescent proteins was performed using a Zeiss Axiovert 200M materials microscope equipped with darkfield, epifluorescence, a FITC filter set, and a Zeiss AxioCam HRM high resolution digital camera. Images were captured using Zeiss' Axiovision software.

## 2.6 Atomic Force Microscopy

Topographic features on patterned silicon substrates were imaged using a Digital Instruments Dimension 3100 atomic force microscope (Digital Instruments/Veeco Metrology Group, Inc.; Santa Barbara, CA) with SiN (DNP-S) probes.

## 2.7 Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

ToF-SIMS measurements were conducted on a PHI-TRIFT III instrument (Physical Electronics USA; Chanhassen, MN) equipped with a gallium liquid metal ion gun (Ga LMIG). The ion gun was operated at either 25kV (unbunched mode) for high image resolution, or 15kV (bunched mode) for high mass resolution. Analyses were done utilizing Ga<sup>+</sup> ions at room temperature. ToF-SIMS measurements were conducted over a 100 x 100  $\mu\text{m}$  area for 10 min. The positive mass spectra were calibrated using common hydrocarbon fragment peaks at CH<sub>3</sub><sup>+</sup>, C<sub>2</sub>H<sub>3</sub><sup>+</sup>, and C<sub>4</sub>H<sub>7</sub><sup>+</sup>, while the negative mass spectra were calibrated using CH<sup>-</sup>, OH<sup>-</sup>, C<sub>2</sub>H<sup>-</sup>. Spectra for background controls were acquired by analyzing clean silicon areas on the wafers.

## 2.8 Proteins and Cell Culture

FITC-neutravidin (Molecular Probes; Eugene, OR) was dissolved in phosphate buffered saline (PBS) at a working concentration of 50  $\mu\text{g}/\text{ml}$ . Photocatalytically patterned samples exposed to FITC-neutravidin were stored in polystyrene dishes sealed with parafilm and wrapped in aluminum foil to keep out light. Dishes were placed on a shaker table for 60 min. Substrates subsequently were rinsed 3 times in PBS, then rinsed in distilled water and dried before imaging.

HeLa cells (Cambrex; East Rutherford, NJ) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies Inc.; Gaithersburg, MD), that was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin/fungizone (antibiotic, Life Technologies). Cultures were maintained in T25 flasks (Corning; Corning, NY) inside an incubator held at 37C and 5% CO<sub>2</sub>. Cells were removed from flasks with Trypsin (Life Technologies) and re-suspended in supplemented DMEM. Cells were counted with a hemocytometer, diluted if necessary, and plated onto substrates at approximate cell densities of 10,000 to 100,000 cells/ml.

### 3. Results

#### 3.1 Surface Modification and Characterization

We demonstrate porphyrin-based photocatalytic lithography using silane patterning as a foundation on which a non-fouling polymer network may be grafted to facilitate biomedical research. Use of unsaturated silanes provides convenient synthetic routes to covalent modification, such as the free radical polymerization techniques described here. We have also grafted thermoreversible polymers (poly(N-isopropylacrylamide), NiPAAm) to patterned silane substrates (results not shown), and we have covalently coupled bacteria to patterned aminosilane coated substrate surfaces (in preparation). Furthermore, we have photocatalytically patterned PLL-PEG (<sup>16, 17</sup>, results not shown), thiols<sup>18</sup>, and PPSPEG(<sup>19, 20</sup>, in preparation).

Atomic Force Microscopy provided topographic and deflection measurements (Fig. 1).

Comparing plane data from a region of the unpatterned matrix and with the patterned L features indicated an IPN thickness of 17 nm, consistent with previous ellipsometric results<sup>14</sup>. Figure 2 illustrates the homogeneity of the coating and the patterned elements.

Time-of-flight Secondary Ion Mass Spectrometry (Fig. 2) confirmed chemical patterning via selected m/z fragment analysis. Note that while PDMS did present surface contamination on a few samples examined by ToF-SIMS, it is a useful material for our process, as it is transparent to radiation at the wavelengths employed, can act as a lens material <sup>21-23</sup>, is reusable and contains large amounts of oxygen to facilitate radical formation. We are presently examining the use of other mask materials, including a polyolefin elastomer by Dow, POP<sup>24</sup>, polyimide (Luxel, Inc.) and polyethylene.

ToF-SIMS indicated that the IPN is characterized by intense hydrocarbon fragments at m/z 13, 15, and 27 (CH, CH<sub>3</sub>, C<sub>2</sub>H<sub>3</sub>), acrylamide related fragments at 26 and 42 (CN, CNO) and PEG related fragments at 41, 43, 45 (C<sub>3</sub>H<sub>5</sub>, C<sub>2</sub>H<sub>3</sub>O, C<sub>2</sub>H<sub>5</sub>O). Peaks due to contamination of sodium contamination (m/z = 23), calcium (39), potassium (40), and PDMS (73) were present on some samples. The oxidatively patterned silicon regions are characterized by intense silicon and oxygen containing fragments, including Si, SiH<sup>+</sup>,

$\text{CH}_3\text{Si}^+$ ,  $\text{SiO}_2$  ( $m/z = 28, 29, 43, 60$ ). Figure 2 includes a selection of positive ion imaging peaks for the AAm/Si layer and then the IPN/APS layers. Contrast is similar in most of the images. However, the total ion image on the IPN/APS substrate shows less contrast, as does the CN peak at 27. While it is unclear why the contrast in the total ion image decreases, we believe that the CN imaging presents less contrast in the IPN/APS sample than the AAm/Si sample because of the nitrogen present on the spots after backfilling with the amino containing silane. The bottom of Figure 2 shows the high resolution spectral analysis window from  $m/z$  28-45 to convey the Si, hydrocarbon and PEG fragments.

### 3.2 Biomolecular adsorption

As shown in Figure 3, fluorescently-tagged protein, FITC-neutravidin provided the first biological test for photocatalytically patterned silicon. A large area (over  $1 \text{ cm}^2$  in total area) was patterned successfully with resolution on the micron-scale in just a few seconds. Protein selectively adsorbed onto the amino-terminated APS-coated Si where ATC had been removed, and protein was repelled from matrix regions where the IPN had been built up. This process worked equally well for both silicon and glass substrates.

Figure 4 shows the result of a HeLa cell plating experiment. Adhesion of the single cells or cell clusters was limited to regions of the adhesive chemistry, as confirmed through optical microscopy. Although the goal of these experiments did not include cell culture longevity, we note that the IPN chemistry (photolithographically patterned) has been proven to maintain cell patterns for up to 60 days<sup>2</sup>. We have carried out cell transfection experiments on photocatalytically patterned substrates and can prove cell viability and successful transfection over a period of at least 5 days<sup>25</sup>. Depending on the goals and targeted substrate materials for experiments, PLL-g-PEG<sup>16</sup> or PPS-bl-PEG<sup>19</sup> can also be photocatalytically patterned to provide similar protein resistance for biotechnological use.

## 4. Discussion

This publication describes initial results of a novel, porphyrin-based technique for patterning surface chemistry, which has applications in a broad variety of settings. Our initial work demonstrates the technique's usefulness in life-science applications. Porphyrins, comprised of four pyrrole residues that are linked by four methine bridging groups formulating an aromatic macrocyclic ring, absorb light energy in order to carry out chemical reactions<sup>26</sup>. They are nature's most prominent catalysts and carry out a spectrum of bioenergetic reactions, ranging from the photosynthetic energy transduction that converts absorbed light in green chlorophyll pigment to usable energy, to the biochemical transductions responsible for oxygen storage and transport throughout the body in hemoglobin and myoglobin, to the conversion of carbon dioxide into hydrocarbons. Porphyrins (and, more generally, photosensitizers) have a long history of use in the field of photodynamic therapy (PDT), treatments that use light to induce beneficial reactions within patients. For a review, see Moan.<sup>27, 28</sup>.

We employ and excite porphyrins to create radical species that photocatalytically oxidize, and thereby pattern, chemistries in the local vicinity. Advantageously, photosensitizer excitation does not necessarily destroy the photosensitizer, which may return to its ground state. Thus, the photosensitizer may repeat such an energy transfer numerous times, which means that multiple patterned surfaces may be made from a single porphyrin mask.

The previously-discussed articles from Tatsuma *et al.* and Kubo *et al.*<sup>11, 12</sup> report excitation times of 20 min for metallic oxide photo-oxidation on substrates to take place. Here, we demonstrate localized oxidation via Type I and Type II photosensitizer reactions on the order of seconds.

In comparison to photolithographic patterning techniques, which are relatively laborious and require resist deposited across the entire substrate surface, photocatalytic patterning may be accomplished on base chemical layers applied to pristine, well controlled, pinhole free surfaces. The process shares some similarities with microcontact printing in that mask materials are brought into close proximity/contact with substrates. However, our process does not rely on mass transport to the surface. For a review on pattern stability via photolithographic, microcontact printed and SMAP methodologies employing PLL-g-PEG chemistry under cell culture conditions see<sup>29</sup>.

Our process has similarities to the TiO<sub>2</sub> based lithography reported first by Tatsuma et al.<sup>11</sup> in that reactive oxygen species are created upon light excitation of catalyst and these reactive oxygen species can decompose underlying chemistries. Tatsuma reported the placement of a polyimide spacer between a quartz mask coated with anatase TiO<sub>2</sub> and an oxidizable surface (silane on Si); light shown through a photomask on top of the quartz plate was used to pattern surface substrate chemistry. A Hg-Xe lamp of 100 mWcm<sup>-2</sup> was employed for at least 8 min before patterning was noted. Lower light intensities (10 mWcm<sup>-2</sup>) required on the order of 30 min to pattern surfaces. The resolution of the technique is dependent on the height of the spacer; small gaps of approximately 10 μm result in resolution on the order of 5 μm<sup>12</sup>. Lee and Sung reported success in patterning silanes down to 500 nm using TiO<sub>2</sub> as a photocatalyst and a 450W Xe lamp for 2 min. In contrast, we can pattern in just a few seconds, using photosensitizer coated PDMS photomasks and significantly longer wavelength light sources with power densities less than 1 mWcm<sup>-2</sup>. While lower wavelengths, such as UV, still effectively stimulate the photosensitizers, it is not clear that higher energy sources decrease patterning time. In fact, UV visible spectrometry indicates that PDMS photomasks show absorption in the UV, and this may increase time necessary to pattern.

## 5. Conclusions

We have presented an inexpensive, rapid, straightforward and versatile method of photocatalytically patterning surface chemistry using porphyrins to generate ablative oxygen radicals. This method is flexible with respect to substrate and chemistry, and is not limited by mass transport. We selectively grafted a non-fouling polymer to remaining unsaturated matrix chemistry. Substrates were analyzed by AFM and Tof-SIMS and tested with fluorescent proteins and cells. Protein adsorption and cell adhesion was restricted to patterned regions, which had been modified with an adhesive chemistry. We intend to

exploit and expand this capability to the nano-scale and demonstrate nanometer patterning resolution with large aspect ratio features.

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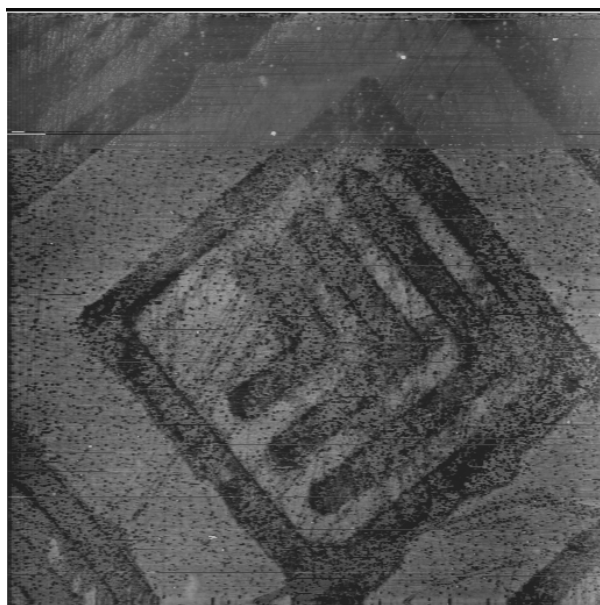


Figure 1. Contact mode AFM image of P(AAm-*co*-EG)/Si substrate patterned with a LLNL logo photomask. Allyltrichlorosilane (ATC) coated substrate was patterned photocatalytically with MgPC and a blue LED. A hydrogel layer, P(AAm-*co*-EG) was then photopolymerized onto remaining silane.

Substrate was sonicated in water and then blown dry with nitrogen before imaging. Height of the hydrogel is on the order of 20 nm. Line width of the Ls is 4  $\mu\text{m}$ .

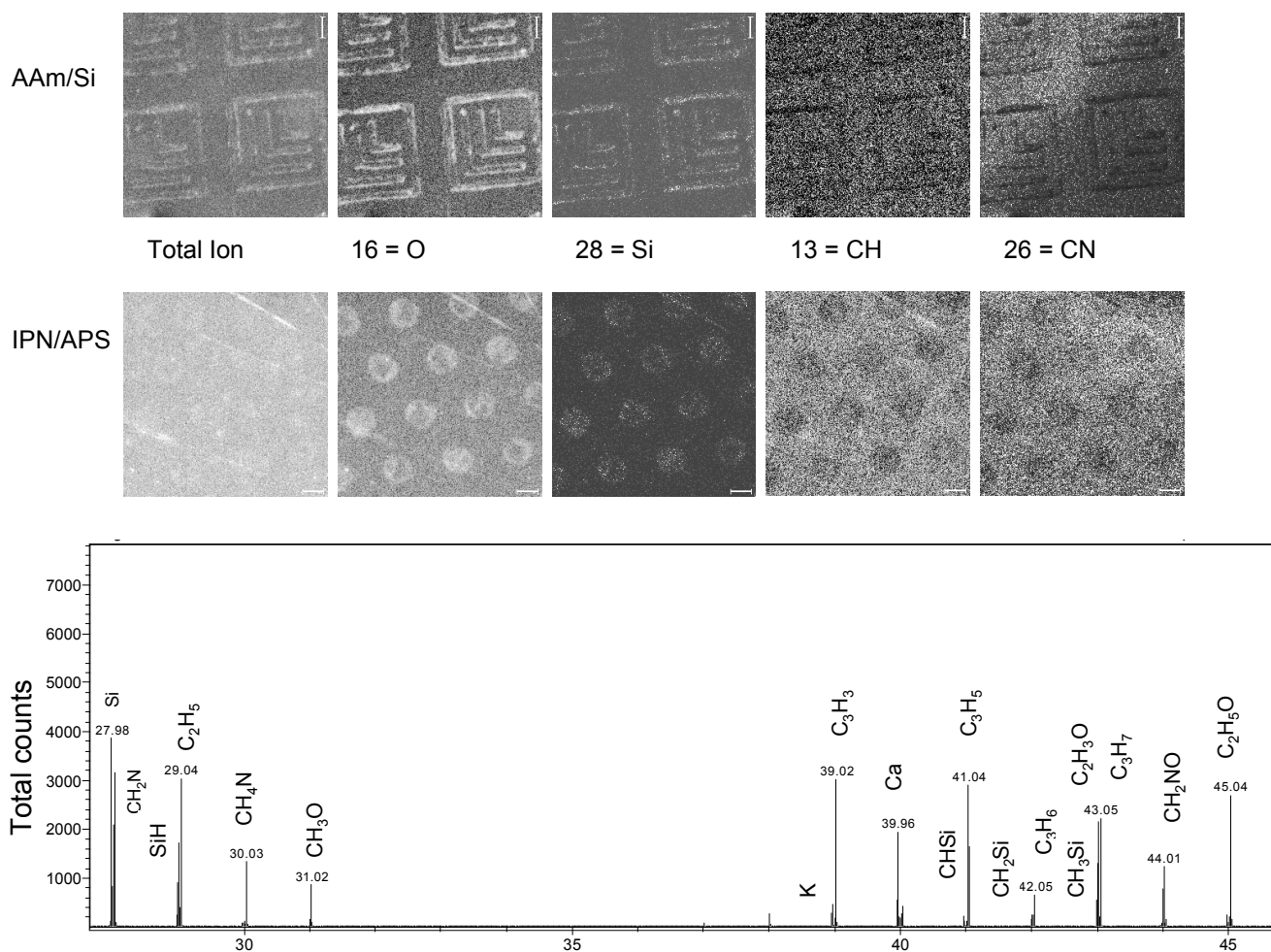


Figure 2. Tof-SIMS conveys chemical proof of patterning. Excerpts of Tof-SIMS positive ion imaging data for AAm/Si patterned substrate (top) and P(AAm-*co*-EG)/aminopropylsilane (IPN/APS, middle). Images include total ion, as well as  $m/z$  ratios indicative of O, Si, CH, and CN. The bottom spectrum shows high resolution data from positive ion imaging of P(AAm-*co*-EG). Counts vs  $m/z$  for the window of 28 to 45 conveys peaks to confirm presence of Si, hydrocarbons and PEG fragments. Scale bars are 5  $\mu\text{m}$ .

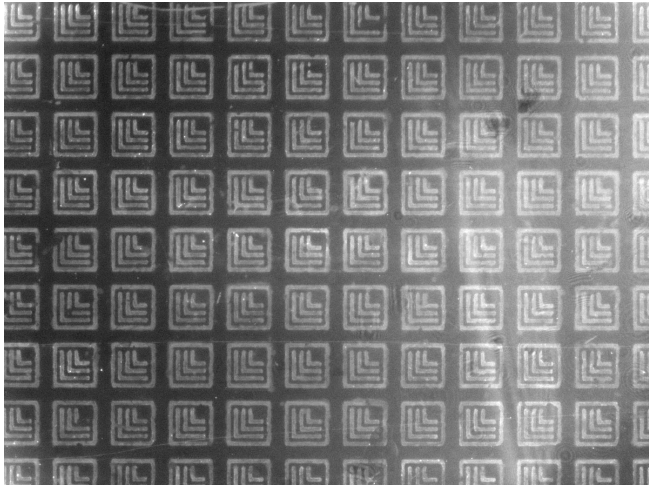
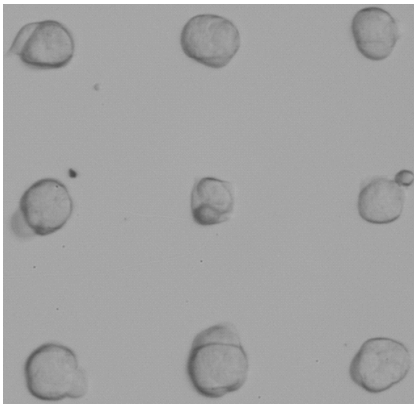


Figure 3. A photomask bearing LLNL logos was used to pattern ATC on silicon. A non-fouling polymer layer (IPN) was then synthesized on the patterned ATC on silicon. After back-filling bare silicon regions with aminopropylsilane (APS), the substrate was incubated with a solution of fluorescein-labeled Neutravidin. The fluorescence micrograph shows that protein selectively adsorbs to APS regions, and is repelled by the non-fouling polymer (IPN) regions (20 $\times$  magnification, line width = 4  $\mu$ m).

A



B

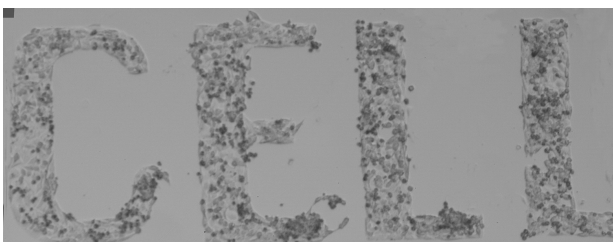
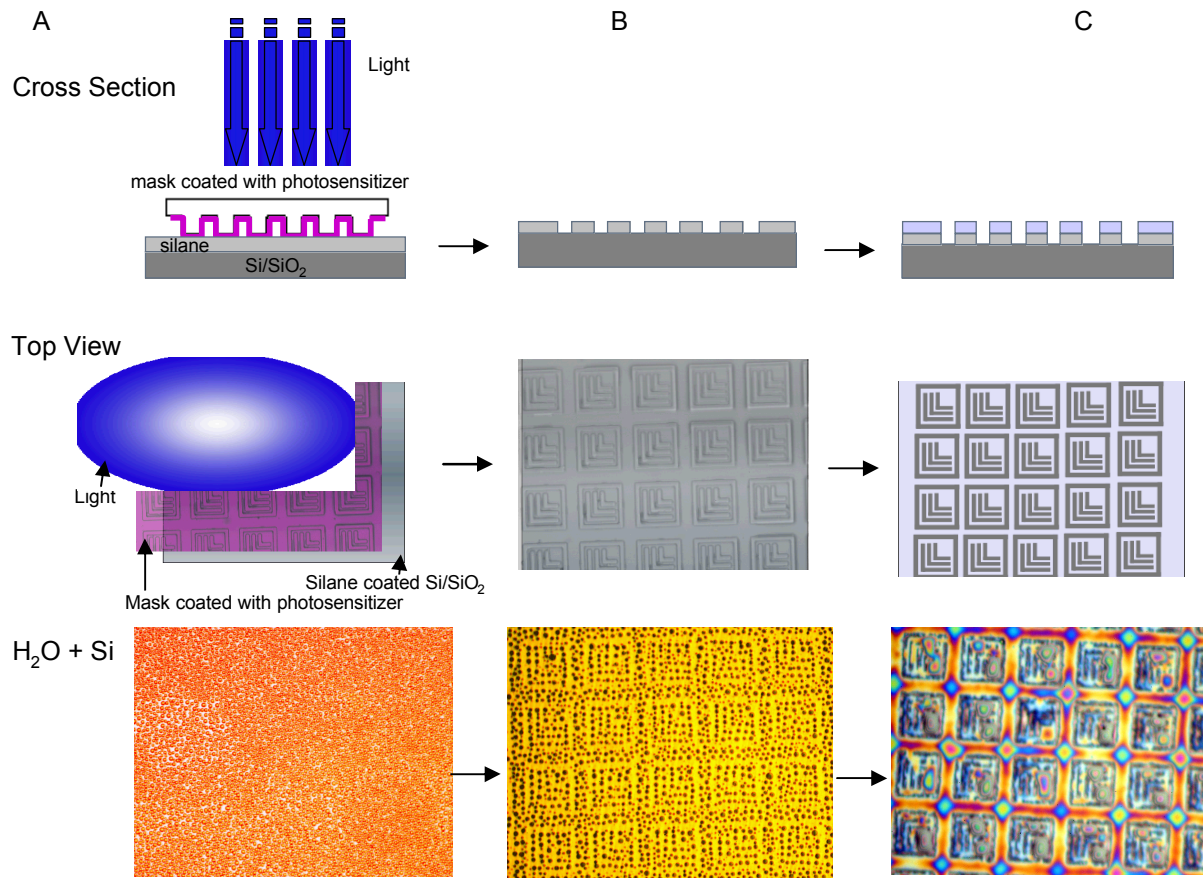


Figure 4. Individual and cell cluster patterning results. Images show HeLa cells plated on IPN/APS photocatalytically patterned substrates: (A) 30  $\mu\text{m}$  circles; (B) letters with line width of 200  $\mu\text{m}$ .

**Scheme 1.** Schematic of photocatalytic patterning process.



Cross section (top), top down view (middle) and hydrated result after substrate exposure to water vapor (bottom): (A) patterning process performed through PDMS photomask coated with photosensitizer from volatile solvent onto silane coated silicon substrate; (B) patterned silane substrate upon selective silane removal from regions subjected to chemical decomposition by reactive oxygen species from excited photosensitizer; (C) polymer grafting of thin acrylamide hydrogel layer onto remaining silane. Ls remain as  $\text{Si}/\text{SiO}_2$  substrate.

Table 1: Absorption peaks for photosensitizers as determined by UV/VIS spectroscopy.

| Photosensitizer                       | Solvent  | Primary Abs. Peak (nm)                            | Secondary Abs. Peak (nm) |
|---------------------------------------|----------|---|--------------------------|
| Chlorophyllin<br>copper sodium salt   | Ethanol  | 411   | 633                      |
| Hematoporphyrin<br>IX dihydrochloride | Methanol | 417   | 559                      |
| Magnesium<br>Phthalocyanine           | Acetone  | Slowly increases from 350 nm<br>(m= -7.82308E-05) |                          |
| Magnesium<br>Phthalocyanine           | Methanol | Slowly decreases from 350 nm<br>(m= 7.38426E-05)  |                          |

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